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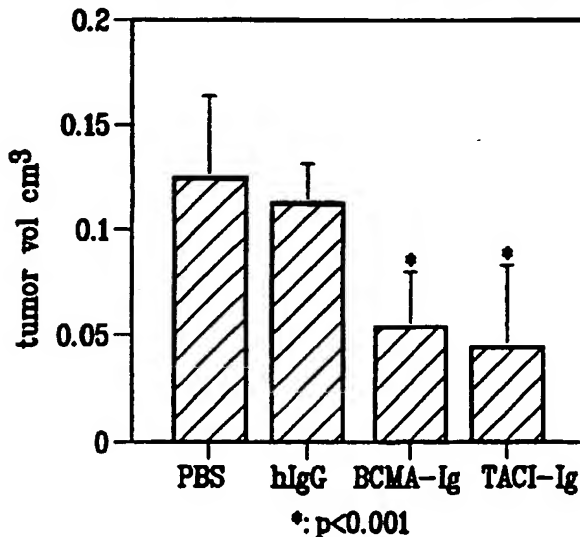
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(54) Title: TACI AS AN ANTI-TUMOR AGENT

Tumor growth 41 days after implantation of A549 lung carcinoma cells sc into nu/nu mice



(57) Abstract: A method of treating a mammal for a condition associated with undesired cell proliferation comprising administering to said mammal an effective amount of an effective amount of a TACI reagent, wherein said reagent extends mean survival time of said mammal by about 10 % or more as compared to the absence of administering the TACI reagent.

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TACI AS AN ANTI-TUMOR AGENT

FIELD OF THE INVENTION

The present invention relates generally to methods of treating a mammal for a condition associated with undesired cell proliferation, including cancer.

BACKGROUND OF THE INVENTION

Members of the tumor-necrosis factor (TNF) family of cytokines are involved in an ever-expanding array of critical biological functions. Each member of the TNF family acts by binding to one or more members of a parallel family of receptor proteins, namely, the TNF receptor family of proteins. TNF receptors, which in turn, signal intracellularly to induce a wide range of physiological or developmental responses. Many of the TNF receptor signals influence cell fate, and often trigger terminal cellular differentiation. Examples of cellular differentiation include proliferation, maturation, migration, and death.

In U.S. patent number 5,969,102, incorporated by reference herein, TACI (Transmembrane Activator CAML Interactor protein), a novel TNF family member receptor protein, is described. The corresponding TNF family member ligand to TACI, however, was not known.

The present invention discloses that the TNF family member APRIL, which is described in applicants co-pending international application PCT/US98/19191, is a ligand to TACI. It is also a discovery of the present invention that TACI reagents are particularly useful in treating a mammal for a condition associated with undesired cell proliferation, including for example, cancer.

SUMMARY OF THE INVENTION

The present invention is directed to a method of treating a mammal for a condition associated with undesired cell proliferation comprising administering to a mammal an effective amount of a TACI reagent. Conditions associated with undesired cell proliferation include but are not limited to cancer and specifically renal cell cancer, Kaposi's sarcoma,

prostate cancer, breast cancer, sarcoma, ovarian carcinoma, rectal cancer, throat cancer, melanoma, colon cancer, bladder cancer, mastocytoma, lung cancer, mammary adenocarcinoma, pharyngeal squamous cell carcinoma, gastrointestinal cancer, and stomach cancer.

In preferred embodiments, the present invention relates to methods of treating a mammal for conditions associated with undesired cell proliferation wherein such cell proliferation is associated with solid tumors. In particular, such solid tumor cancerous conditions include tumors of the prostate, lung, breast, colorectal, bladder, endometrium, ovary, oropharynx/larynx, cervix, stomach, pancreas, and the brain (and central nervous system).

Also contemplated are methods for reducing the size of a tumor located on or in a mammal comprising administering to said mammal an effective amount of a TACI reagent. In preferred embodiments, the tumor is a solid tumor.

The methods of the present invention include the use of a fusion protein comprising at least two segments, wherein a first segment comprises a substantially pure TACI protein or polypeptide fragment thereof, and a second segment comprises an immunoglobulin polypeptide. The immunoglobulin polypeptide is preferably a human IgG Fc domain.

It is to be understood from the foregoing general description and the following detailed description are exemplary and explanatory, and are intended to provide further explanation of the invention claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are included to provide a further understanding of the invention, and are incorporated in, and constitute a part of this specification, illustrate several embodiments of the invention, and together with the description serve to explain the principles of the invention.

Figure 1 is a schematic representation of the nucleic acid sequence (SEQ ID NO:2) of a cDNA for human TACI and its derived amino acid sequence (SEQ ID NO:1) as mapped in vector pCA336.

Figure 2 is a schematic representation of the nucleic acid sequence insert in pJST552 encoding N-terminus FLAG-tagged human full length TACI, and its derived amino acid sequence.

Figure 3 is a schematic representation of the DNA sequence (SEQ ID NO:5) and its derived amino acid sequence (SEQ ID NO:6) of the TACI extracellular domain with a truncated stalk region fused to human IgG Fc. This was assembled as plasmid pJST572. The signal sequence from murine IgG-kappa, nucleotides 1-69 (amino acids 1-23), was fused in frame with the human TACI extracellular domain (amino acids 1-114 of SEQ ID NO:1) as nucleotides 70-411 (amino acids 24-137) which was fused in frame to the hIgG1 Fc as nucleotides 412-1098 (amino acids 138-366). The predicted signal peptidase cleavage site is after amino acid 20.

Figure 4 is a schematic representation of the DNA sequence (SEQ ID NO:7) and its derived amino acid sequence (SEQ ID NO:8) of the TACI extracellular domain with a truncated stalk region initiating after the second methionine fused to human IgG Fc. This was assembled as plasmid pJST591. The signal sequence from murine IgG-kappa, nucleotides 1-66 (amino acids 1-22), was fused in frame with human TACI extracellular domain (amino acids 32-114 of SEQ ID NO:1) as nucleotides 67-315 (amino acids 23-105) which was fused in frame to the hIgG1 Fc as nucleotides 316-1002 (amino acids 106-334). The predicted signal peptidase cleavage site is after amino acid 20.

Figure 5 is a schematic representation of the nucleic acid sequence and its derived amino acid sequence of the complete extracellular domain of TACI fused to a human IgG-Fc sequence, as assembled in plasmid PS882, wherein there is a short hemagglutinin (HA)-signal sequence in frame with the native methionine (amino acid 18) and TACI extracellular domain sequence through amino acid 177 (valine, which is amino acid 160 of TACI) after which there is a human IgG-Fc construct in frame.

Figure 6 is a schematic representation of the nucleic acid sequence (SEQ ID NO:4) and its derived amino acid sequence (SEQ ID NO:3) of a myc-tagged murine APRIL construct for expression in *Pichia pastoris* cells, as mapped in plasmid pCMM276, including the alpha mating factor signal sequence, which is cleaved off; the myc epitope (first 11 amino acids after the signal sequence; underlined); a short linker region (next 8 amino acids); and the soluble extracellular domain of murine APRIL coding sequence from amino acid 20, which is an alanine, to the first stop codon.

Figure 7 is a schematic representation of the nucleic acid sequence of FLAG-tagged soluble extracellular domain of murine APRIL, and the corresponding amino acid sequence, as mapped in the mammalian expression plasmid PS784, also known as LT032, wherein there is

an HA-signal sequence (boxed), the FLAG epitope (underlined), a short linker sequence, then soluble murine APRIL sequence (arrow beginning at alanine).

Figure 8 is a schematic representation of purified myc-tagged murine APRIL binding to TACI transfected cells. 293EBNA cells were transfected with expression plasmid pJST552 that expressed FLAG-tagged full length human TACI. Cells were harvested 48 hours later using 5 mM EDTA and stained with myc-tagged murine APRIL at various concentrations. No protein control was stained with detection reagents (rabbit anti-murine APRIL and donkey anti-rabbit-PE (Jackson Immunoresearch)). Staining in FL1 of protein encoded by the cotransfected GFP expression plasmid illustrates expression efficiency.

Figure 9 is a schematic representation of the DNA sequence of FLAG tagged soluble extracellular domain of human APRIL (SEQ ID NO:9) and the corresponding amino acid sequence (SEQ ID NO:10) as cloned in the mammalian expression vector LT033. This construct contains an HA signal sequence tag (boxed), the FLAG epitope tag (underlined), and a short linker sequence fused to soluble human APRIL (arrow, beginning at alanine).

Figure 10 is a series of representations of Western blots delineating the immunoprecipitation of FLAG-tagged murine APRIL using human TACI(1-160)-Ig (hTACI(1-160)-Ig) fusion protein. Figure 10A is a representation of a Western blot showing Ponceau-S staining of protein loads for the ligands. Figure 10B is a representation of a Western blot showing the amount of hTACI(1-160)-Ig used in the immunoprecipitations by revealing the IgG-Fc portion. Figure 10C is a representation of a Western blot showing that only APRIL immunoprecipitated with hTACI(1-160)-Ig, as evidenced by revealing the FLAG-tag.

Figure 11 shows the results of *in vivo* tumor growth inhibition of HT29 colon adenocarcinoma cells by hTACI(1-114)-Ig.

Figure 12 shows the results of *in vivo* tumor growth inhibition of A594 lung carcinoma cells by hTACI(1-114)-Ig.

Figure 13 shows a histogram overlay of hTACI(1-114)-Ig and hTACI(32-114)-Ig binding to cells stably expressing surface murine APRIL.

Figure 14 shows an plot of an ELISA analysis of showing binding of murine and human APRIL to hTACI(32-114)-Ig.

DETAILED DESCRIPTION OF THE INVENTION

The patent applications, patents and literature references cited herein indicate the knowledge of those of ordinary skill in this field and are hereby incorporated by reference in their entirety. In the case of inconsistencies between any reference cited herein and the specific teachings of the present disclosure, this disclosure will prevail. Similarly, any inconsistencies between an art-understood meaning of a term and a meaning of a term as specifically taught in the present disclosure will be resolved in favor of this disclosure.

It has now been unexpectedly discovered that TACI is a receptor for APRIL (A Proliferation Inducing Ligand). It is also an unexpected discovery of the present invention that TACI reagents can be used to treat a mammal for a condition associated with undesired cell proliferation comprising administering to said mammal an effective amount of a TACI reagent, wherein said reagent extends mean survival time of said mammal by about 10 %, 15%, 20%, 25% or more compared to the absence of administering the TACI reagent for said condition. Moreover, it is an unexpected discovery of the present invention that TACI reagents can be used to reduce the size of a tumor located on or in a mammal comprising administering to said mammal an effective amount of a TACI reagent, wherein said reagent reduces the size of said tumor by about 10 %, 15%, 20%, 25% or more as compared to not administering the TACI reagent.

As used herein, "treating or treatment" means an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviation of symptoms, diminishment of extent of disease, stabilized (*e.g.*, not worsening) state of disease, preventing spread (*e.g.*, metastasis) of disease, preventing occurrence or recurrence of disease, delay or slowing of disease progression, amelioration of the disease state, and remission (whether partial or total). Also encompassed by "treatment" is a reduction of pathological consequences of a condition associated with undesired cell proliferation, including specifically, cancer.

By "mammal" as used herein means any mammal including humans, cows, horses, dogs, rats, mice and cats. In preferred embodiment of the invention, the mammal is a human.

"A condition associated with undesired cell proliferation" as used herein includes but is not limited to cancer, specifically, conditions comprising at least one solid tumor, including, but not limited to renal cell cancer, Kaposi's sarcoma, breast cancer, sarcoma, ovarian carcinoma, rectal cancer, throat cancer, melanoma, colon cancer, bladder cancer,

mastocytoma, lung cancer, mammary adenocarcinoma, pharyngeal squamous cell carcinoma, gastrointestinal cancer or stomach cancer. Preferably, the cancer is mastocytoma, melanoma, lymphoma, mammary adenocarcinoma, prostate and breast cancer. Also contemplated are other conditions associated with undesired cell proliferation including but not limited to cellular hyperproliferation (hyperplasia), selected from the group consisting of, for example, scleroderma, pannus formation in rheumatoid arthritis, post-surgical scarring and lung, liver, and uterine fibrosis.

As used herein "administering" means the TACI reagent can be administered alone or in combination with other pharmaceutical agents and can be combined with a physiologically acceptable carrier therefor. The effective amount and method of administration of the particular TACI reagent can vary based on the individual mammal and the stage of the disease and other factors evident to one skilled in the art. The route(s) of administration useful in a particular application are apparent to one of skill in the art.

Routes of administration include but are not limited to topical, transdermal, parenteral, gastrointestinal, transbronchial and transalveolar. Topical administration is accomplished via a topically applied cream, gel, rinse, etc. containing an oligonucleotide conjugate. Transdermal administration is accomplished by application of a cream, rinse, gel, etc. capable of allowing the TACI reagent to penetrate the skin and enter the blood stream. Parenteral routes of administration include but are not limited to electrical or direct injection such as direct injection into a central venous line, intravenous, intramuscular, intraperitoneal or subcutaneous injection. Gastrointestinal routes of administration include but are not limited to ingestion and rectal administration. Transbronchial and transalveolar routes of administration include but are not limited to inhalation, either via the mouth or intranasally.

An "effective amount" as used herein is an amount sufficient to effect beneficial or desired clinical results (Stites *et al.*, BASIC & CLINICAL IMMUNOLOGY, Lange Medical Publications, Los Altos, California, 1982). An effective amount can be administered in one or more administrations as described herein. For purposes of this invention, an effective amount of a TACI reagent is an amount sufficient to extend mean survival time of a mammal by at least 10%, alternatively 15%, 20% or 25% in comparison to mean survival in the absence of administering a TACI reagent. Detection and measurement of indicators of efficacy are generally based on measurement of clinical symptoms associated with the disease state, such as increased average life expectancy after treatment with a TACI reagent.

An effective amount of a TACI reagent for reducing the size of a tumor in or on a mammal is an amount sufficient to reduce tumor size on or in a mammal by at least 10%, alternatively 15%, 20% or 25% more than in the absence of administering a TACI reagent. Methods for measuring tumor size in a mammal are known to those of skill in the art and can be measured by non-invasive procedures, including but not limited to using a micrometer to measure the tumor diameter, if the tumor is located on the exterior surface of a mammal. Alternatively if the tumor is located in the interior of the mammal one can use MRI to measure the tumor diameter. Invasive procedures include surgically removing the tumor from the mammal and weighing the tumor and comparing the size of the tumor to pretreatment with the TACI reagent.

As used herein "a TACI reagent" means those reagents that can influence how the TACI signal is interpreted within the cell including antagonistic TACI reagents that can diminish ligand binding to TACI, including for example, TACI fusion proteins such as TACI-IgG Fc. Also contemplated are agonistic TACI reagents that can augment ligand binding to TACI, including for example, antibodies to TACI such as anti-TACI monoclonal antibodies. The term Fc domain refers to a part of the molecule comprising the hinge, CH2 and CH3 domains, but lacking the antigen binding sites. The term is meant to include the equivalent regions of an IgG, an IgM and other antibody isotypes.

Another aspect of the invention relates to the use of the polypeptide encoded by the isolated nucleic acid encoding TACI in "antisense" therapy. As used herein, "antisense" therapy refers to administration or *in situ* generation of oligonucleotides or their derivatives which specifically hybridize under cellular conditions with the cellular mRNA and/or DNA encoding the ligand of interest, so as to inhibit expression of the encoded protein, *i.e.*, by inhibiting transcription and/or translation. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix. In general, "antisense" therapy refers to a range of techniques generally employed in the art, and includes any therapy that relies on specific binding to oligonucleotide sequences.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid, which, when transcribed in the cell, produces RNA that is complementary to at least a portion of the cellular mRNA which encodes TACI. Alternatively, the antisense construct can be an oligonucleotide probe that is generated *ex vivo*. Such oligonucleotide probes are preferably modified oligonucleotides that are resistant to endogenous nucleases,

and are, therefore, stable *in vivo*. Exemplary nucleic acids molecules for use as antisense oligonucleotides are phosphoramidates, phosphothioate and methylphosphonate analogs of DNA (See, e.g., U.S. Patent Nos. 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy have been reviewed, for example, by Van Der Krol *et al.*, (1988) *Biotechniques* 6:958-976; and Stein *et al.* (1988) *Cancer Res.* 48:2659-2668, specifically incorporated herein by reference. In some embodiments the antisense oligonucleotides are complementary to a regulatory region of the mRNA that encodes TACI. In other embodiments, the antisense oligonucleotides are complementary to a protein encoding portion of the mRNA encoding TACI. In some embodiments of the invention the antisense oligonucleotides are about 12 to about 35 nucleotides in length. In other embodiments, the antisense oligonucleotides are about 15 to about 25 nucleotides in length. In other embodiments, the antisense oligonucleotides are about 17 to about 22 nucleotides in length.

As used herein, "extend mean survival time" means that the average life expectancy associated with a particular condition associated with undesired cell proliferation is on average increased. Average life expectancy is known to those of skill in the art for various forms of cancer in various forms of mammals, including various forms of cancer in humans, and various forms of cancer in rodents, including mice. Furthermore, as used herein, an extended mean survival time of, for example, about 10% or more as compared to mean survival time in the absence of administering a TACI reagent, means for example, that for a human patient with a form of cancer that has an survival time of about 365 days (1 year) in the absence of treatment, a TACI reagent would increase their average life expectancy by about 10% of 365 days or more, for a total of about 400 days total survival.

By "soluble TACI reagent" means a soluble form of a TACI protein or polypeptide fragment in which the transmembrane domain has been cleaved or mutated by standard biochemical or recombinant DNA techniques such that it is soluble.

In another aspect, the invention provides a method of treating a mammal for a condition associated with undesired cell proliferation comprising administering to the mammal an effective amount of a substantially pure, soluble form of a TACI protein or polypeptide fragment of a TACI protein, wherein the TACI protein or polypeptide fragment of the TACI protein binds the extracellular domain of APRIL and thereby inhibits aberrant cell growth. By "protein" or "polypeptide" means any molecule comprising two or more amino acids joined

together with a peptide bond, regardless of length or post-translational modifications (*e.g.*, glycosylation, lipidation, acetylation, or phosphorylation).

As used herein, by "polypeptide fragment" means a polypeptide that is shorter in length than the full length protein from which it was derived but greater than a single amino acid. Hence, a polypeptide fragment of a TACI protein has less amino acids than the full length TACI protein. For example "hTACI(1-160)" refers to a human TACI polypeptide sequence containing amino acid residues 1 through 160 of human TACI. "hTACI(1-114)" refers to a human TACI polypeptide sequence containing amino acid residues 1 through 123 of human TACI. "hTACI(32-114)" refers to a human TACI polypeptide sequence containing amino acid residues 32 through 123 of human TACI. In embodiments in which a TACI fragment is joined as a fusion protein to a portion of an immunoglobulin molecule, "-Ig" is added to the end of the designation for the TACI fragment (*e.g.*, "hTACI(1-160)-Ig," "hTACI(1-114)-Ig," and "hTACI(32-114)-Ig") to indicate the fusion protein.

A preferred polypeptide fragment of a TACI protein is the soluble extracellular domain of TACI. For example, a preferred polypeptide fragment of a TACI protein is, without limitation, amino acids 1 to about 166 (*e.g.* 1 to about 161 or 1 to about 171) and other amino acids in between. Other preferred polypeptide fragments include, but are not limited to amino acids 1 to about 114 and amino acids from about 32 to about 114. A preferred polypeptide fragment of TACI is one that binds to the extracellular domain of APRIL.

The claimed invention in certain embodiments includes methods of using peptides derived from TACI which have the ability to bind to APRIL. Fragments of TACI can be produced in several ways, *e.g.*, recombinantly, by PCR, proteolytic digestion or by chemical synthesis. Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end or both ends of a nucleic acid that encodes the polypeptide. Expression of the mutagenized DNA produces polypeptide fragments.

Polypeptide fragments can also be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-moc or t-boc chemistry. For example, peptides and DNA sequences of the present invention may be arbitrarily divided into fragments of desired length with no overlap of the fragment, or divided into overlapping fragments of a desired length. Methods such as these are described in more detail below.

Soluble forms of the TACI can often signal effectively and, hence, can be administered as a drug which now mimics the natural membrane form. It is possible that the TACI claimed herein are naturally secreted as soluble cytokines, however, if not, one can reengineer the gene

to force secretion. To create a soluble secreted form of TACI, one would remove at the DNA level the N-terminus transmembrane regions, and some portion of the stalk region, and replace them with a type I leader or alternatively a type II leader sequence that will allow efficient proteolytic cleavage in the chosen expression system. A skilled artisan could vary the amount of the stalk region retained in the secretion expression construct to optimize both ligand binding properties and secretion efficiency. For example, the constructs containing all possible stalk lengths, *i.e.*, N-terminal truncations, could be prepared. In certain embodiments, proteins starting at amino acids 1 to 32 are produced. The optimal length stalk sequence would result from this type of analysis.

By "substantially pure" or "substantially purified" is meant a compound (*e.g.*, a nucleic acid molecule or a protein) that has been separated from components (*e.g.*, nucleic acid molecules, proteins, lipids, and/or carbohydrates) which naturally accompany it. Water, buffers, and other small molecules (*e.g.*, molecules having a molecular weight of less than about 1000 daltons) may accompany a substantially pure compound of the invention. Preferably, a substantially purified compound is at least 70%, by weight, free from components which naturally accompany it. More preferably, a substantially purified compound is at least 75%, by weight, free from components which naturally accompany it; still more preferably, at least 80%, by weight, free; even more preferably, at least 85%, by weight, free; and even more preferably, at least 90%, by weight, free from components which naturally accompany it. Most preferably, a substantially purified compound is at least 95%, by weight, free from components which naturally accompany it. In certain embodiments of the second aspect of the invention, the polypeptide fragment of the TACI protein has an amino acid sequence that is included within the extracellular domain of TACI. This fragment may be any size that is smaller than the TACI extracellular domain. Thus, this fragment may include from about 26% to about 99% of the extracellular domain, and so may include any part of amino acids 1 to about 166 by SEQ ID NO:1. For example, a polypeptide fragment of the invention includes the N-terminal amino acid residues 1 to about 166 of SEQ ID NO:1. In other embodiments, the polypeptide fragment of the invention includes the N-terminal amino acid residues 1 to about 114 of SEQ ID NO:1. In other embodiments, the polypeptide fragment of the invention includes the N-terminal amino acid residues from about 32 to about 114 of SEQ ID NO:1.

The "TACI extracellular domain" refers to a form of a TACI protein or polypeptide which is essentially free of transmembrane and cytoplasmic domains of TACI. Ordinarily,

TACI extracellular domain have less than 1% of such transmembrane and cytoplasmic domains and preferably have less than 0.5% of such domains. In a preferred embodiment, the TACI extracellular domain is amino acids 1 to about 166 of SEQ ID NO:1. It is understood by the skilled artisan that the transmembrane domain identified for the TACI protein or polypeptide fragment of the present invention is identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain vary but most likely by no more than about five amino acids at either end of the domain specifically mentioned herein.

In accordance with this second aspect, the invention provides all derivative, mutants, truncations, and/or splice variants of TACI, so long as these derivatives, mutants, truncations, and/or splice variants share at least 26% amino acid sequence identity with SEQ ID NO:1, preferably, at least 30% sequence identity, more preferably at least 50% sequence identity, more preferably at least 65% sequence identity, more preferably, at least 70% sequence identity, more preferably, at least 75% sequence identity, still more preferably, at least 80% sequence identity, and even more preferably at least 85% sequence identity, and still even more preferably, at least 90% sequence identity, and most preferably, at least 95% sequence identity with SEQ ID NO:1, using the sequence of the TACI derivative, mutant, truncation, and/or splice variants as the probe. "Sequence identity" with respect TACI amino acid sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the TACI amino acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity is achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN, or Megalign (DNASTAR) software. Those skilled in the art determine appropriate parameters for measuring local alignment, including any algorithms needed to achieve maximum alignment over the full length of the sequences being compared, to detect relationships among sequences which share only isolated regions of similarity. (Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410).

Accordingly, derivative, mutants, truncations, and/or splice variants of a TACI protein displaying substantially equivalent or altered activity are likewise contemplated. These variants may be deliberate, for example, such as modifications obtained through site-directed mutagenesis, or may be accidental, such as those obtained through mutations in hosts that are

producers of the protein. Included within the scope of these terms are proteins specifically recited herein, as well as all substantially homologous analogs and allelic variations.

In one non-limiting example, in accordance with the invention, a soluble, substantially pure TACI protein consisting essentially of amino acid residues 1 to about 166 of SEQ ID NO:1, or soluble variations thereof, is chemically synthesized according to standard techniques (*e.g.*, at a commercial peptide generating facility). Alternatively, a soluble, substantially pure TACI protein or polypeptide fragment thereof, is synthesized by standard, well-known recombinant DNA techniques in prokaryotic or eucaryotic host cells.

Analogues of TACI can differ from the naturally occurring TACI in amino acid sequence, or in ways that do not involve sequence, or both. Non-sequence modifications include *in vivo* or *in vitro* chemical derivatization of TACI. Non-sequence modifications include, but are not limited to, changes in acetylation, methylation, phosphorylation, carboxylation or glycosylation.

Preferred analogues include TACI, biologically active fragments thereof, whose sequences differ from the sequence given in SEQ ID NO:1, by one or more conservative amino acid substitutions, or by one or more non-conservative amino acid substitutions, deletions or insertions which do not abolish the activity of TACI. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics (*e.g.*, substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and, phenylalanine, tyrosine).

In another aspect, the invention encompasses a method of treating cancer in a mammal comprising administering an effective amount of a soluble, substantially pure fusion protein comprising a soluble form of a TACI protein or polypeptide fragment thereof, wherein the fusion protein inhibits cell growth. In certain embodiments, the TACI fusion protein has an amino acid sequence comprising the extracellular domain of SEQ ID NO:1, or a portion of the extracellular domain.

By "fusion protein" means a protein that comprises at least two segments of a protein or polypeptide fragment joined together by any means, including, without limitation, a covalent bond (*e.g.*, peptide bond), a non-covalent bond (*e.g.*, ionic bond or hydrogen bond) or by a chemical crosslinker. Also, any variety of fusion proteins carrying only the extracellular domain of the TACI protein can be generated. Non-limiting examples include a fusion protein

comprising the extracellular domain of the TACI protein and an immunoglobulin polypeptide, including for example, the immunoglobulin polypeptide IgG.

The invention also includes antibodies specifically reactive with the claimed TACI reagents. Anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols (See, for example, ANTIBODIES: A LABORATORY MANUAL Harlow and Lane, Eds., Cold Spring Harbor Press, N.Y., 1988). A mammal such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the peptide. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers, or other techniques, well known in the art.

An immunogenic portion of TACI can be administered in the presence of an adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies.

In a preferred embodiment, the subject antibodies are immunospecific for antigenic determinants of TACI, *e.g.*, antigenic determinants of a polypeptide of SEQ ID NO:1, or a closely related human or non-human mammalian homolog (*e.g.*, 70, 80 or 90 percent homologous, more preferably at least 95 percent homologous). In yet a further preferred embodiment of the present invention, the anti-TACI antibodies do not substantially cross react (*i.e.*, react specifically) with a protein which is for example, less than 80 percent homologous to SEQ ID NO:1; preferably less than 90 percent homologous with SEQ ID NO:1; and, most preferably less than 95 percent homologous with SEQ ID NO:1. By "not substantially cross react," it is meant that the antibody has a binding affinity for a non-homologous protein which is less than 10 percent, more preferably less than 5 percent, and even more preferably less than 1 percent, of the binding affinity for a protein of SEQ ID NO.1.

The term antibody as used herein is intended to include fragments of antibodies which are also specifically reactive with TACI. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab')₂ fragments can be generated by treating antibody with pepsin. The resulting F(ab')₂ fragment can be treated to reduce disulfide bridges to produce Fab' fragments. The antibodies of the present invention are further intended to include biospecific and chimeric molecules having anti-TACI activity. Thus, both monoclonal and polyclonal antibodies (Ab) directed against TACI, and antibody fragments such as Fab' and F(ab')₂, can be used to block the action of the TACI.

Various forms of antibodies can also be made using standard recombinant DNA techniques. (Winter and Milstein, (1991) *Nature* 349:293-299, specifically incorporated by reference herein). For example, chimeric antibodies can be constructed in which the antigen binding domain from an animal antibody is linked to a human constant domain (e.g., U.S. Patent No. 4,816,567, to Cabilly *et al.*, incorporated herein by reference). Chimeric antibodies may reduce the observed immunogenic responses elicited by animal antibodies when used in human clinical treatments.

In addition, recombinant "humanized antibodies" which recognize TACI can be synthesized. Humanized antibodies are chimeras comprising mostly human IgG sequences into which the regions responsible for specific antigen-binding have been inserted. Animals are immunized with the desired antigen, the corresponding antibodies are isolated, and the portion of the variable region sequences responsible for specific antigen binding are removed. The animal-derived antigen binding regions are then cloned into the appropriate position of human antibody genes in which the antigen binding regions have been deleted. Humanized antibodies minimize the use of heterologous (*i.e.*, inter species) sequences in human antibodies, and thus are less likely to elicit immune responses in the treated subject.

Construction of different classes of recombinant antibodies can also be accomplished by making chimeric or humanized antibodies comprising variable domains and human constant domains (CH1, CH2, CH3) isolated from different classes of immunoglobulins. For example, antibodies with increased antigen binding site valencies can be recombinantly produced by cloning the antigen binding site into vectors carrying the human heavy chain constant regions. (Arulanandam *et al.*, (1993) *J. Exp. Med.* 177:1439-1450, incorporated herein by reference).

In addition, standard recombinant DNA techniques can be used to alter the binding affinities of recombinant antibodies with their antigens by altering amino acid residues in the vicinity of the antigen binding sites. The antigen binding affinity of a humanized antibody can be increased by mutagenesis based on molecular modeling. (Queen *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:10029-10033) incorporated herein by reference.

The present invention also provides pharmaceutical compositions comprising a TACI polypeptide and a pharmaceutically acceptable excipient. Suitable carriers for a TACI polypeptide, for instance, and their formulations, are described in REMINGTON' PHARMACEUTICAL SCIENCES, 16th ed., Oslo *et al.* Eds., Mack Publishing Co., 1980. Typically, an appropriate amount of a pharmaceutically acceptable salt is used in the formulation to

render the formulation isotonic. Examples of the carrier include buffers such as saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7.4 to about 7.8. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers, which matrices are in the form of shaped articles, *e.g.*, liposomes, films, or microparticles. It will be apparent to those of skill in the art that certain carriers may be more preferable depending upon for instance the route of administration and concentration of the TACI polypeptide being administered.

Administration may be accomplished by injection (*e.g.*, intravenous, intraperitoneal, subcutaneous, intramuscular) or by other methods such as infusion that ensure delivery to the bloodstream in an effective form.

In another aspect of the invention, the invention is directed to a method of treating a mammal for a condition associated with undesired cell proliferation, comprising administering to the mammal an effective amount of a substantially pure binding agent that specifically binds a APRIL protein, wherein the binding of the binding agent to APRIL inhibits undesired cell proliferation. The binding agent in this aspect is a protein having at least 26% sequence identity with amino acid residues 1 to about 166 of SEQ ID NO:1. Preferably, the binding agent shares at least 30% sequence identity, more preferably at least 50% sequence identity, more preferably at least 65% sequence identity, more preferably, at least 70% sequence identity, more preferably, at least 75% sequence identity, still more preferably, at least 80% sequence identity, and even more preferably at least 85% sequence identity, and still even more preferably, at least 90% sequence identity, and most preferably, at least 95% sequence identity with SEQ ID NO:1, using the sequence of the TACI derivative, mutant, truncation, and/or splice variant as the probe. In certain embodiments, the APRIL protein has an amino acid sequence described in WO 99/12965. In certain preferred embodiments, the binding agent is an antibody, such as a polyclonal antibody, or a monoclonal antibody, or a recombinant, humanized, or chimeric antibody, or a fragment of an antibody that specifically binds an APRIL protein or a extracellular domain thereof.

The TACI reagents are administered in an effective amount which may easily be extrapolated by the animal data provided herein by methods known to those of ordinary skill in the art (*e.g.*, based on body weight, body surface area). Furthermore, it is in the purview of the skilled physician to increase or decrease amounts of the TACI reagent to achieve the desired effects without causing any undesirable side effects.

The following Examples are provided to illustrate the present invention, and should not be construed as limiting thereof.

EXAMPLES

The following methods and materials were used in the Examples disclosed hereinafter.

I. Methods and Materials

A. Cloning and expression of myc-tagged A88 murine APRIL in *Pichia pastoris*

The expression vector pCCM276 (Figure 4), was constructed by polymerase chain reaction using pCCM213.10 (Myc-tagged-H98 muAPRIL) as template and synthetic oligonucleotides CDL620 and LTB619 as forward and reverse primers, respectively. Forward primer CDL620 adds back 10 amino acids of murine APRIL sequence to represent the native cleaved form, along with a FAS ligand derived KEL motif and SacI site. Resultant amplified fragments were digested with *SacI* and *NotI*, gel purified and ligated into those same restriction sites of pCCM213.10. This expression construct contains yeast signal sequence alpha mating factor directly fused to myc epitope tag, KEL motif and murine APRIL starting at Alanine 88. pCCM276 was linearized with *StuI*, electroporated into GS115 strain (his4-) and plated onto minimal media containing dextrose. HIS4 transformants were analyzed for protein expression by inoculating a single representative colony in rich media (BMGY) and allowing it to grow to density for 48 hours at 30°C. Cultures were spun, and cell pellets were resuspended (1:5) in a rich induction media containing 2% methanol (BMMY). After two days of induction at 30°C, supernates were run out on SDS-PAGE and assessed for the presence of muAPRIL. Coomassie blue staining and Western Blot (with the anti-myc mAB 9E10) analysis confirmed the presence of the glycosylated A88 myc-tagged murine APRIL.

B. Myc-Murine APRIL A88 Purification

Myc-tagged murine April (myc-muAPRIL) was expressed in *P. pastoris*. The protein has an isoelectric point of about 7.45. 175 ml of *Pichia* supernate was dialyzed into PBS overnight with a 30kD cutoff dialysis membrane. The dialysate was then passed through a Q sepharose column. The myc-muAPRIL was collected in the column flow through, while contaminants were bound to the column. The eluted myc-muAPRIL was concentrated 20 fold and then chromatographed on a superdex 75 gel filtration column. After gel filtration, 8 mg of myc-muAPRIL were recovered having an OD of 1.0AU-1 mg of myc-muAPRIL. Coomassie

stained SDS PAGE showed a homogenous preparation of myc-muAPRIL with two bands migrating at molecular weights of approximately 22 kD and 18 kD. Western blot analysis using mouse monoclonal 9E10 antibody (anti-myc) showed that both bands observed were immunoreactive. The expected N-terminus of the purified myc-muAPRIL was verified by Edman degradation of the blotted protein. The N-terminal sequencing and immunoreactivity to 9E10 prove the myc tag was present on the myc-muAPRIL N-termini.

C. FLAG-Murine APRIL Purification

Plasmid PS784 (also called LT032) (Figure 7) was used to transiently transfect 293 T cells using lipofectamine reagent (Gibco-BRL) and serum free media. The plasmid, constructed in the mammalian expression vector PCR3 (Invitrogen) encodes the soluble receptor-binding domain of murine APRIL, with an N-terminal FLAG-tag, into the cell culture media. FLAG-APRIL protein was purified from serum free media using an anti-FLAG mAb M2 column and excess purified FLAG peptide, following the manufacturers' instructions (Kodak). Alternatively murine APRIL and other FLAG-tagged ligands were analyzed directly from conditioned media.

D. hTACI(1-160)-Ig Expression

A plasmid encoding soluble hTACI(1-160)-Ig (PS882; Figure 5) was used to transiently to transfect 293 cells. Conditioned media from 293 cells overexpressing hTACI(1-160)-Ig was used in the immunoprecipitation analysis to detect binding to APRIL protein.

E. Full Length TACI Expression

A plasmid encoding flag-tagged full length TACI (pJST552; Figure 2) was transiently transfected into 293 cells. The full length form of the molecule is retained on the cell surface. These transfected cells were used in FACS analyses to detect binding to APRIL protein.

F. FLAG-human APRIL production

Plasmid LT033 (Figure 9) was used to transiently transfect 293 T cells using Lipofectamine reagent (Gibco-BRL) and serum-free media. The plasmid, constructed in mammalian expression vector PCR3 (Invitrogen) encodes the soluble receptor-binding domain of human APRIL, with an N-terminal FLAG-tag, and the expressed protein is secreted into the cell culture media. FLAG human APRIL was analyzed directly from cell culture supernate.

EXAMPLE 1

Human TACI Expressed on Cells Interacts With Myc-Tagged Murine APRIL

293EBNA cells were co-transfected with a plasmids expressing full length N-terminally FLAG tagged human TACI (pJST552)(see Figure 2), and a GFP marker, (AN050). Transfections were performed using Lipofectamine 2000 (LifeTechnologies) according to manufacturer's conditions. Transfection media was DMEM supplemented with 10% FBS, 4 mM glutamine, and 50 μ M Z-VAD (BACHEM Bioscience Inc.) Cells were harvested with PBS supplemented with 5 mM EDTA at 24 hours post-transfection. Cells were washed in FACS buffer (PBS-10% FBS-0.02% NaN_3) and resuspended at a density of 5×10^6 cells/mL. Expression of TACI was verified by staining 100 microliters of transfected cells with anti-FLAG mAb at 5 μ g/ml on ice for 30 minutes followed by donkey anti-mouse IgG-PE at 1:100 (Jackson ImmunoResearch). Cells were assayed for their ability to bind APRIL over a concentration range of 3 μ g/ml to 300 ng/ml by incubating on ice in FACS buffer for 30 minutes. Binding was revealed using Rb1532, a rabbit polyclonal Ab raised to mAPRIL (1:100), followed by donkey anti-rabbit IgG-PE (1:100, Jackson ImmunoResearch). 7-AAD was included in the terminal stain and used to gate out dead cells. The samples were analyzed by FACS and plotted (Figure 8). Cell gate analyzed is shown as R1. Specific staining is seen at the three different concentrations of myc-tagged murine APRIL protein shown, as compared to no protein control (Figure 8).

EXAMPLE 2

Soluble hTACI(1-160)-Ig Interacts with FLAG-Tagged Murine APRIL

250 μ l of Optimem containing hTACI(1-160)-Ig was mixed with various FLAG ligands of the TNF family. Some of the ligands were in Optimem while others were anti-FLAG (mAb M2)-purified. The common characteristic was that the amount used was empirically the same (500 ng for purified ligands, *i.e.* hBAFF, muBAFF, TRAIL, FasL, EDA). Ligands were produced in bacteria (hBAFF, TRAIL) or transiently expressed in 293 EBNA cells (all others). Control Receptors-Fc were mostly used as Optimem supernates, except TNFR1, OX40, LTBR, which were purified over protein-A columns. 1 μ g of purified receptors was used. Receptor-Fc proteins (about 1 μ g) were mixed with FLAG-ligands (about 500 ng). Volume was adjusted to 1.2 ml with PBS and 5 μ l of protein A-Sepharose was added. Incubation was performed for 1 h at 4°C on a wheel. Beads were harvested by centrifugation and loaded onto a minicolumn (yellow tips with 1 mm diameter frit). Washes

were performed by applying vacuum at the bottom of the column to aspirate medium and 2 times 400 μ l PBS washes. The dried beads were eluted with 20 μ l of Citrate -NaOH pH 2.7 and the eluate was neutralized with 6 μ l of 1M Tris-HCl pH 9.0. 10 μ l 3x sample buffer plus DTT was added, the sample was boiled and 22 μ l loaded for Western blot analysis. The membrane was sequentially stained with Ponceau-S, blocked in 4% milk, 0.5% Tween-20, then incubated with 1 μ g/ml M2 in blocking buffer, washed, and revealed with goat anti-mouse-Ig-peroxidase (1/5000 in block buffer). ECL was used to illuminate the signal. Peroxidase activity was removed with azide, the membrane washed, and then probed again with donkey anti-human-peroxidase.

The western results of the co-immunoprecipitation show that only flag-muAPRIL is able to be co-immunoprecipitated by TACI(1-160)-Ig. The binding of TACI(1-160)-Ig to muAPRIL appears to be specific as none of the other 14 flag tagged soluble TNF family ligands were able to interact with TACI(1-160)-Ig.

EXAMPLE 3

Use of Derived TACI Sequence, and Antagonists and Agonists of TACI Binding and Activity, as Modifiers of Oncological and Neoplastic Disorders

Examples of the preparation and inoculation of transformed cells to assess tumor cell growth is described in Celis *et al.*, CELL BIOLOGY, A LABORATORY HANDBOOK, Volume One, Academic Press, San Diego, CA. 1997. Tumor cells were derived from a variety of sources, including the extensive tumor cell banks maintained by ATCC (Bethesda, MD), immortalized cell lines, immortalized primary cell lines, stably transfected cell lines, tumor tissue derived from mammalian sources, including humans. Tissue source is a major determinant of choice of immunodeficient or normal animals (Celis *et al.*, *ibid.*). In addition there are a wide variety of techniques for the induction of tumor growth in various animal models using carcinogenic or other insults.

Models which utilize tumor growth in immunodeficient mice as a means of readily determining the activity of novel ligands on tumor biology (Hahne *et al.* (1998) *J. Exp. Med.* 188:1185-1190) were developed. Both ligands and their antagonists were assayed in such systems, *e.g.*, Kashii *et al.* (1999) *J. Immunol.* 163:5358-5366; Zhai *et al.* (1999) *FASEB J.* 13:181-189). An agonist mAb to a TNF receptor family member (LTBR) profoundly affected tumor cell growth and survival was demonstrated (Browning *et al.* (1996) *J. Exp. Med.* 183:867-878).

Tumor cell lines were implanted in immunodeficient mice subcutaneously, and the growth rate of tumors in mice treated with TACI-Ig was similar to the growth rate of tumors in mice treated with approximately 5mg/kg/week CBE11, that is, much slower tumor growth as compared to the growth rate of mice given control treatments.

Another variation of these models was to use as a tumor source cells known to induce metastasis in immunodeficient or syngeneic animals. For example, the ATCC (Bethesda, MD) provided numerous human tumor lines with known metastatic potential to a variety of tissues, and these lines are utilized to examine the effect of TACI activity or antagonism on metastasis. These models have been and are now currently used (*e.g.*, by the NCI) to assess the potential for treatment of human patients.

EXAMPLE 4

Generation of Soluble TACI Receptors

To form a receptor inhibitor for use in man, the human receptor cDNA sequence of the extracellular domain of TACI was used (SEQ ID NO:2). With a human cDNA sequence, oligonucleotide primers were designed to PCR amplify the extracellular domain of the receptor in the absence of the transmembrane and intracellular domains. Typically, one of ordinary skill included most of the amino acids between the last disulfide linked "TNF domain" and the transmembrane domain. Alternatively, the amount of "stalk" region was varied to optimize the potency of the resultant soluble receptor. This amplified piece was engineered to include suitable restriction sites to allow cloning into various C-terminal Ig fusion chimera vectors. Alternatively, a stop signal at the 3' end was inserted to make a soluble form of the receptor without resorting to the use of an Ig fusion chimera approach. The resultant vectors were expressed in most systems used in biotechnology including yeast, insect cells, bacteria and mammalian cells and examples exist for all types of expression. Various human Fc domains were attached to optimize or eliminate FcR and complement interactions as desired. Alternatively, mutated forms of these Fc domains were used to selectively remove FcR or complement interactions or the attachment of N-linked sugars to the Fc domain which has certain advantages.

EXAMPLE 5**Screening for Inhibitors of the Receptor-Ligand Interaction**

Using the receptor-Ig fusion protein, one screens either combinatorial libraries as screened for molecules that bind the receptor directly. These molecules are then tested in an ELISA formatted assay using the receptor-Ig fusion protein and a soluble form of the ligand for the ability to inhibit the receptor-ligand interaction. This ELISA is used directly to screen various natural product libraries, *etc.* for inhibitory compounds. The receptor is transfected into a cell line such as the HT29 line to form a biological assay (in this case cytotoxicity) that then form the screening assay to further demonstrate blocking.

EXAMPLE 6**In vivo Tumor Growth Inhibition**

The number of tumor cells injected subcutaneously (s.c.) can be determined in titration studies prior to initiating work with antagonists. For example, the SW480 – colon adenocarcinoma solid tumor line and NIH 3T3 fibrosarcoma line, which grow aggressively, 8×10^5 cells and 5×10^6 cells, respectively, can be implanted in nude mice. Dosing with control or TACI-Ig proteins can begin just prior to implantation, with subsequent doses every 7 days thereafter. The dose can be for example 100 μ g/mouse. Tumor diameter is then measured using a micrometer, and the volume is calculated using the formula $\text{vol} = 4/3\pi r^3$.

EXAMPLE 7**In vivo Tumor Growth Inhibition**

The number of tumor cells injected s.c. into immunodeficient (nu/nu) mice was determined in previous dose response studies. For the studies using HT29, 1×10^6 cells were implanted per mouse. HT29 is derived from a human colon adenocarcinoma, and has similar growth characteristics to some other human colon adenocarcinoma lines (*eg.*, SW480) such as rapid tumor formation, and rapid tumor growth. Mice implanted with HT29 cells were treated on the day of implantation, and every week thereafter, with 100 μ g hTACI(1-114)-Ig given intraperitoneally (i.p.). Negative controls included the irrelevant proteins polyclonal hIgG and mAb MOPC21. Positive controls included BCMA-Ig, another inhibitor of APRIL binding, and CBE11, a mAb to the mLTb-R which is known to slow adenocarcinoma tumor growth (Browning *et al.* (1996) *J. Exp. Med.* 183:867-878). Tumor diameter was measured using a micrometer, and the volume was calculated using the formula $\text{vol} = 4/3\pi R^3$.

For studies with the lung carcinoma A549 we implanted 1×10^6 cells/mouse. Mice were treated with 100 μ g TACI(1-114)-Ig, 100 μ g BCMA-Ig, 100 μ g hIgG, or 100 μ l PBS starting on the day of implantation and treated weekly thereafter. Tumor diameter was measured using a micrometer, and the volume was calculated using the formula $\text{vol} = 4/3\pi R^3$.

The results showing tumor growth inhibition of the HT29 colon adenocarcinoma are shown in Figure 11. The results showing tumor growth inhibition of the A549 lung carcinoma are shown in Figure 12. In both experiments, significant slowing of tumor growth was achieved. Since tumor size and survival are directly linked in these models, TACI-Ig treatment also impacted animal survival. For example, 95 days after tumor implantation, 50% of the hIgG treated mice were scored as terminal, compared to only 12.5% of the TACI-Ig treated mice. This represents a 4 fold increase in survival at this end stage time point.

EXAMPLE 8

hTACI(1-114)-Ig and hTACI(32-114)-Ig bind to surface localized APRIL

This example shows titratable binding of TACI-Ig fusion proteins a stable cell line expressing muAPRIL on their surface. Either 25 μ l or 5 μ l of conditioned media from 293EBNA cells transiently transfected with plasmids expressing hTACI(1-114)-Ig, hTACI(32-114) or hFN14-Fc were diluted in FACS buffer to a final volume of 50 μ l and incubated for 1 hour on ice with 2.5×10^5 293 cells stably expressing the receptor binding domain of muAPRIL on their surface. After washing with FACS buffer, cells were then incubated with anti-human IgG-PE (1:100 dilution, Jackson ImmunoResearch) for 30 minutes on ice. Cells were again washed, fixed in 1% paraformaldehyde and analyzed by FACS. Figure 13 shows an FL2 shift indicating titratable staining of surface muAPRIL by hTACI(1-114)-Ig and hTACI(32-114)-Ig. No staining was observed with the second step only control. No staining was observed with either dilution of a control-Ig, FN14-Ig fusion protein, both of which co-migrate with the second step only histogram.

EXAMPLE 9

Binding of murine and human APRIL to hTACI(32-114)-Ig in ELISA format

ELISA plates (Corning) were coated with 5 μ g/ml hTACI(32-114)-Ig or mLTbR-Ig control in bicarbonate buffer pH = 9.6 overnight, then washed in PBS/0.05% Tween-20 solution before blocking for 2 h at 37°C with PBS/2% bovine serum albumin (BSA). APRIL protein preparations were added in the range of 0.0048 to 3 μ g/ml, diluted in PBS/2% BSA.

Detection of specific APRIL binding was with rabbit anti-murine APRIL antisera (R1532) and donkey anti-rabbit HRP (Jackson ImmunoResearch) or with HRP-coupled anti FLAG mAb M2 (Kodak). Enzymatic development was done with TMA and H₂O₂ and stopped with H₂SO₄, following standard protocols. The developed yellow stain was read at 450 nm on a plate reader. The results are shown in Figure 14.

EXAMPLE 10

Preferential Expression of APRIL in Tumor Samples

APRIL mRNA is preferentially expressed in tumor samples. Analysis of the cDNA libraries collected by Incyte Inc., and shown in Table I, demonstrates that APRIL is widely expressed in numerous solid tumor and neoplastic samples, but much less often expressed in normal tissue samples. Expression in diseased tissue is occasionally prominent.

Table I

EXPRESSION OF APRIL IN TISSUES (INCYTE)		
PROSTATE/BLADDER	No. +	DISTRIBUTION
Normal	5	
Tumor	11	adenocarcinoma
COLON		
Normal	0	
Tumor	6	adenocarcinoma
Diseased	4	2 Crohn's Disease 1 ulcerative colitis 1 polyp
BREAST		
Normal	1	normal epithelium
Tumor	11	5 adenocarcinoma 3 ductal carcinoma 1 lobule carcinoma 2 unspecified
PANCREAS		
Normal	0	
Tumor	5	1 anaplastic carcinoma 4 adenocarcinoma
LUNG		
Normal	2	4 adenocarcinoma
Tumor	12	4 adenocarcinoma 3 squamous cell carcinoma 3 carcinomoid spindle cell endobonchial neuroendocrine

		2 metastases
Diseased	7	
UTERUS/OVARY		
Normal	0	
Tumor	13	3 cystadenocarcinoma 2 endometrial tumors 4 leiomyomata 2 adenocarcinoma 1 papillary carcinoma 1 metastases
Diseased	1	1 ovarian cyst
Other Normal Tissues		
MO/DC	10	
Endothelial	5	2 arterial endothelium 1 dermal endothelium 1 arterial smooth muscle 1 arterial plaque
Other Hyperplastic Sites	6	2 lymphomas 2 lymphoid hyperplasia 2 rheumatoid arthritis synovium 1 hyperthyroid 1 nasal polyp

It is apparent to those skilled in the art that various modifications and variations are made in the methods of the invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided that they come within the scope of the appended claims and their equivalents.

What is claimed is:

1. A method of treating a mammal for a condition associated with undesired cell proliferation comprising administering to said mammal an effective amount of a TACI reagent that extends mean survival time of said mammal as compared to the mean survival time of said mammal in the absence of administering said TACI reagent, wherein said undesired cell proliferation comprises at least one solid tumor.
2. The method of claim 1, wherein said TACI reagent extends mean survival time of said mammal by about 15%.
3. The method of claim 1, wherein said TACI reagent extends mean survival time of said mammal by about 20%.
4. The method of claim 1, wherein said TACI reagent extends mean survival time of said mammal by about 25%.
5. The method of claims 1, 2, 3 or 4, wherein said TACI reagent is a fusion protein comprising at least two segments, wherein a first segment comprises a substantially pure TACI protein or polypeptide fragment thereof, and a second segment comprises an immunoglobulin polypeptide.
6. The method of claim 5, wherein the immunoglobulin polypeptide is an Fc domain.
7. The method of claim 6, wherein said Fc domain is an IgG Fc domain.
8. The method of claim 5 wherein said first segment of said fusion protein is selected from the group consisting of:
 - (a) the extracellular domain of a TACI polypeptide;
 - (b) amino acid residues 1-166 of SEQ ID NO:1;
 - (c) amino acid residues 1-160 of SEQ ID NO:1;
 - (d) amino acid residues 1-114 of SEQ ID NO:1; and
 - (e) amino acid residues 32-114 of SEQ ID NO:1.

9. The method of claim 5 wherein said first segment of said fusion protein is selected from the group consisting of:

- (a) a polypeptide having at least 80% amino acid sequence identity with the extracellular domain of a TACI polypeptide;
- (b) a polypeptide having at least 80% amino acid sequence identity with amino acid residues 1-166 of SEQ ID NO:1;
- (c) a polypeptide having at least 80% amino acid sequence identity with amino acid residues 1-160 of SEQ ID NO:1;
- (d) a polypeptide having at least 80% amino acid sequence identity with amino acid residues 1-114 of SEQ ID NO:1; and
- (e) a polypeptide having at least 80% amino acid sequence identity with amino acid residues 32-114 of SEQ ID NO:1.

10. The method of claims 1, 2, 3 or 4 wherein the said condition associated with undesired cell proliferation is a cancer selected from the group consisting of renal cell cancer, Kaposi's sarcoma, breast cancer, sarcoma, ovarian carcinoma, rectal cancer, throat cancer, melanoma, colon cancer, bladder cancer, mastocytoma, lung cancer, mammary adenocarcinoma, pharyngeal squamous cell carcinoma, gastrointestinal cancer, and stomach cancer.

11. The method of claims 1, 2, 3 or 4, wherein the mammal is selected from the group consisting of a human, a cow, a horse, a dog, a mouse, a rat, and a cat.

12. A method of reducing the size of a solid tumor located on or in a mammal comprising administering to said mammal an effective amount of a TACI reagent, wherein said reagent reduces the size of said solid tumor by about 10% or more.

13. The method of claim 12, wherein said TACI reagent reduces the size of said tumor by about 15% or more.

14. The method of claim 12, wherein said TACI reagent reduces the size of said tumor by about 20% or more.

15. The method of claim 12, wherein said TACI reagent reduces the size of said tumor by about 25% or more.
16. The method of claims 12, 13, 14 or 15 wherein said TACI reagent is a fusion protein comprising at least two segments, wherein a first segment comprises a substantially pure TACI protein or polypeptide fragment thereof, and a second segment comprises an immunoglobulin polypeptide.
17. The method of claim 16, wherein the immunoglobulin polypeptide is an Fc domain.
18. The method of claim 17, wherein said Fc domain is an IgG Fc domain.
19. The method of claim 16 wherein said first segment of said fusion protein is selected from the group consisting of:
- (a) the extracellular domain of a TACI polypeptide;
 - (b) amino acid residues 1-166 of SEQ ID NO:1;
 - (c) amino acid residues 1-160 of SEQ ID NO:1;
 - (d) amino acid residues 1-114 of SEQ ID NO:1; and
 - (e) amino acid residues 32-114 of SEQ ID NO:1.
20. The method of claim 16 wherein said first segment of said fusion protein is selected from the group consisting of:
- (a) a polypeptide having at least 80% amino acid sequence identity with the extracellular domain of a TACI polypeptide;
 - (b) a polypeptide having at least 80% amino acid sequence identity with amino acid residues 1-166 of SEQ ID NO:1;
 - (c) a polypeptide having at least 80% amino acid sequence identity with amino acid residues 1-160 of SEQ ID NO:1;
 - (d) a polypeptide having at least 80% amino acid sequence identity with amino acid residues 1-114 of SEQ ID NO:1; and
 - (e) a polypeptide having at least 80% amino acid sequence identity with amino acid residues 32-114 of SEQ ID NO:1.

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FIG. 2

SacI XbaI KpnI PvuII HindIII NotI
 AGCGAAATGGCGGTAGGCGTGTATACGGTGGGAGGTCTATATTAAGCAGAGCTGTGTAGTGAACCGTCAGATCTCTAGAAGCTGGGTACTCAGCTGTCTAGCAGCTTGTAGCGCGCCGccatggacta
 > M D Y
 junction marker
 caaagacatgacgacaagaagtggccctg99ccggagagagCGCAGGTGGCCGAGCGCTGTGTGACACGAGGAGCGCTTTCCACAGGAGCCCTGTGTACGAGGAGCTGTGTATGAGATCTGTGCCCCGGAAGA
 K D D D D K S G L G R S R R G G R S R V D Q E E R F P Q G L W T G V A M R S C P E E
 BamHI
 GCAGTACTGGGATCTCTGCTGGGTACCTGCATGTCCTGCGAAACCAATTGCAACATCAGAGCCAGCCACCTGTGACGCTTCTGTGAGGTCACTCAGCTGCGCCGCAAGGAGCAAGGCAAGTTCCTA
 Q Y W D C T L G T C M S C K T I C N H Q S Q R T C A A F C R S L S C R K E Q G K P Y
 TGACCATCTCTGAGGAGCTGCATCAGCTGTGCTCCATCTGTGACAGCACCCTAAGCAATGTGCATATCTGTGTGAACAAGCTTCAGGAGCCAGTGAACCTTCCACCAGAGCTCAGGAGACA
 D H L L R D C I S C A S I C G Q H P K Q C A Y F C E N K L R S P V N L P P E L R R Q
 CGGAGTGGAGAGTTGAAAACAATTACAGCAACTCGGGAAGGTACCAAGATTCGAGACAGAGGCTCAGAAAGCAAGTCCAGCTCTCCCGGGCTGTGAAGCTUAGTGCAGATCAGGTGGCCCTGGT
 R S G E V B N N S D N S G R Y Q G L E H R G S E A S P A L P G L K L S A D Q V A L V
 BsmHI
 CTACAGCACGCTGGGGCTCTGCTGTGTGCGGCTCTCTGCTGTGCGGTGGCGCTGTCTTCTCAAGAAAGAGGAGGAGATCTCTGTCTCTGACGCCCCGCTCAAGGCCCGCTCAAGATCC
 Y S T L G G L C L C A V L C C F L V A V A C F L K K R G D P C S C Q P R S R P R Q S P
 GGCAAGCTTTCCCAAGATCAGCGATGGAAGCGGAGCCCTGTGAGCACATCCCGGAGCCAGTGGAGACCTGTCAGCTTCTGTCTCCCTGATGTGAGGCGGCCCAAGCGAGGAGAGCGAGTCAAC
 A K S S Q D H A M E A G S P V S T S P E P V E T C S F P E C R A P T Q E S A V T
 GCTTGGGACCCCGACCCCACTGTGTCTGTGAAGGTGGGGTGGCCACACCAAGATCCAGTCTCTGAGCTTGGCCACACATCCAGACAGATGGCGCTTGGCATTTGTGTGTGCTGTGCCCGCCAGAGAGG
 P G T P D P T C A G R W G C H T R T T V L Q P C P H I P D S G L G I V C V P A Q E G
 NotI XhoI SfiI BamHI
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 G P G A * M G V S G R S R P A
 MunI
 TTGTGAATTTGTGATGCTATGTCTTTATTTGTAAACCAATTATTAAGCTGTGCAATAAACAAGTTTAACAACAACAATTCATTTATTTGTTTTCAGCTTCAAGGGGAGGTGGGAGGTTTTTAAAGC
 AAGTAAACAACCTCTACAAATGTGGTATGGCTGAATTATGATCCGCTGCTCCCTGCGCTTCCGTTCCGTTGATGACGGGTGAACCACTCTGACACATGTCAGCTCCCGGAGACGGTCAACAGCTTGTCTGTAAAGCGG
 XbaI
 ATGCCCGGAGCAGACAAGCCCTCAGGGCGCGTCTAGCGGGTGTGGCGGTGTGGGGGCGCAGCCATGAACCGGTCCACTATAGA

bTACI(1-114)-IgG1

1	ATG	GAG	ACA	GAC	ACA	CTC	CTG	TTA	TGG	GTG	CTG	CTC	TGG	GTT	CCA	GGT	TCC	ACT	GGT	GAC	GTC	ACG	ATG	AGT	GGC	CTG			
1>	M	E	T	D	T	L	L	L	L	W	V	L	L	W	V	P	G	S	T	G	D	V	T	M	S	G	L		
82	GGC	CGG	AGC	AGG	CGA	GGT	GGC	CGG	AGC	CGT	GTG	GAC	CAG	GAG	CGC	TTT	CCA	CAG	GGC	CTG	TGG	ACA	GGG	GTC	GCT	ATG			
28>	G	R	S	R	R	G	G	R	S	R	V	D	Q	E	E	R	F	P	Q	G	L	W	T	G	V	A	M		
163	AGA	TCC	TGC	CCC	GAA	GAG	CAG	TAC	TGG	GAT	CCT	CTG	CTG	GGT	ACC	TGC	ATG	TCC	TGC	AAA	ACC	ATT	TGC	AAC	CAT	CAG	AGC		
55>	R	S	C	P	E	E	Q	Y	W	D	P	L	L	G	T	C	M	S	C	K	T	I	C	N	H	Q	S		
244	CAG	CGC	ACC	TGT	GCA	GCC	TTC	TGC	AGG	TCA	CTC	AGC	TGC	CGC	AAG	GAG	CAA	GGC	AAG	TTC	TAT	GAC	CAT	CTC	CTG	AGG	GAC		
82>	Q	R	T	C	A	F	C	R	S	L	S	L	S	C	R	K	E	Q	G	K	F	Y	D	H	L	L	R	D	
325	TGC	ATC	AGC	TGT	GCC	TCC	ATC	TGT	GGA	CTC	GAC	CAC	CCT	AAG	CAA	TGT	GCA	TAT	TTC	TGT	GAG	AAC	AAG	CTC	AGG	AGC	CCA	GTG	
109>	C	I	S	C	A	S	I	C	G	Q	H	P	C	P	A	Q	C	A	Y	F	C	E	N	K	L	R	S	P	V
406	AAC	CTT	CCA	GTC	GAC	AAA	ACT	CAC	ACA	TGC	CCA	CCG	TGC	CCA	CCT	GAA	CTC	GTG	GGG	GGG	ACA	CGG	TCA	GTG	TTC	CTC	TTC	TTC	
136>	N	L	P	V	D	K	T	H	T	C	P	P	C	P	A	P	E	L	L	G	G	P	S	V	F	L	F	F	
487	CCC	CCA	AAA	CCC	AAG	GAC	ACC	CTC	ATG	ATC	TCC	CGG	ACC	CCT	GAG	GTG	ACA	TGC	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	GTG	
163>	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	V	V	D	V	S	H	E	D	D	
568	CCT	GAG	GTC	AAK	TTC	AAC	TGG	TAC	GTG	GAC	GSC	GTG	GAG	GTG	CAT	AAT	GCC	AAG	ACA	AAG	CCG	CGG	GAG	GAG	CAG	TAC	AAC	AAC	
190>	P	E	V	K	F	N	W	Y	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	N	
649	AGC	ACG	TAC	CGT	GTG	GTC	ATC	ACC	GTG	CTC	ACC	GTG	CAC	GAG	GAC	TGG	CTG	AAT	GGC	AAG	GAG	TAC	AAG	TGC	AAG	GTG	GTG	TCC	
217>	S	T	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S	S	
730	AAC	AAA	GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA	ACC	ATC	TCC	AAA	GCC	AAA	GCC	CAG	CCC	CGA	AAA	CCA	CAG	GTG	TAC	ACC	CTG	CTG	
244>	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K	G	Q	P	R	E	P	Q	V	Y	T	L	L	
811	CCC	CCA	TCC	CGG	GAT	GAG	CTG	ACC	AAG	AAC	CAG	GTG	AGC	CTG	ACC	TGC	CTG	GTG	AAA	GGC	TTC	TAT	CCC	AGC	GAC	ATC	GCC	GCC	
271>	P	P	S	R	D	E	L	T	K	N	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	A	
892	PTG	GAG	TGG	GAG	AGC	AAT	GGG	CAG	CCG	GAG	AAC	AAC	TAC	AAG	ACC	ACG	CCT	CCC	GTG	TTG	GAC	TCC	GAC	GGC	TCC	TTC	TTC	ATC	
308>	V	E	W	E	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D						

FIG. 4

hTACI(32-114)-IgG1 Fc

[illegible]

[illegible]

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FIG. 6

[illegible]

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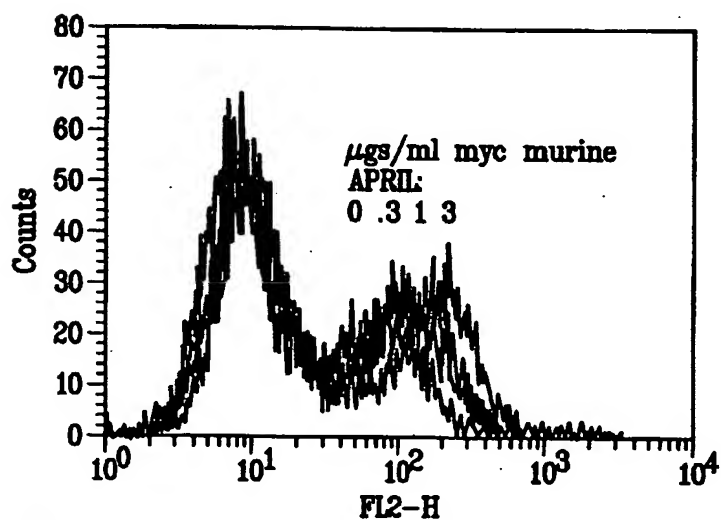
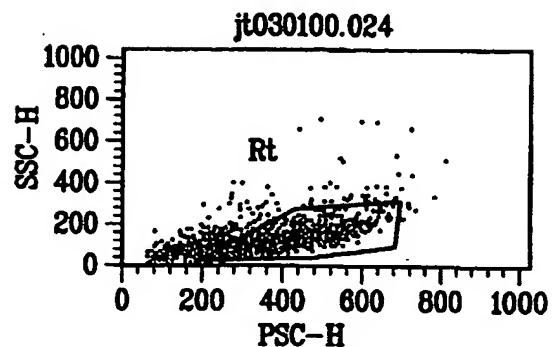
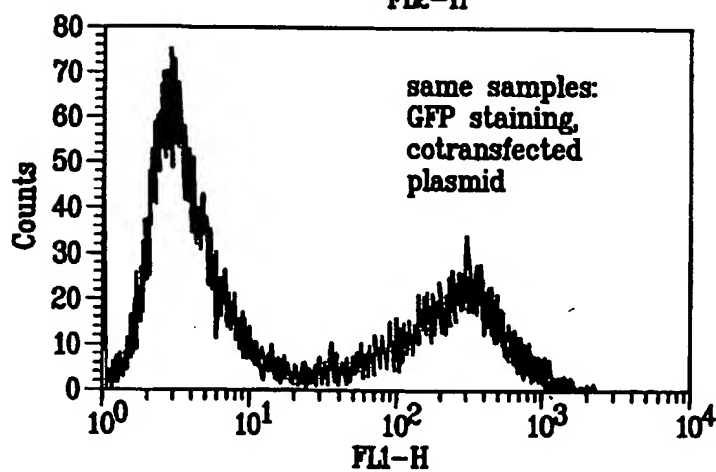
FIG. 7

AAGCTTAATCAAAACATGGCTATCATCTACCTCATCTCTCTGTTCCACCGCTGTGCGGGCGATTACAAGACGATGACGATAAAGGACCCGGACAGGTGCGAGTCACTC
 TTCGAATTACTTTTGTACCGATAGTAGAGGACAGTGGGACACGCCCGCTAATGTTCTGTACTGCTATTCTCTGGGCTGTCCACGTCGAGTCCGTCATGAG
 → M A I I Y L I L L P T A V R G D Y K D D D K G P G Q V Q L Q A V L
 ACCGAGACACAAGAGCACTCAGTCTCTGCTCTGTTCCAGTTAACTTACCTCCAGGACTCTGACGTGACAGAGGTGATGTGGCAACCAAGTACTTAGGCGTGGGAGAGGC
 TGGGTCTTCGTGTTCTTCTCGTAGTCAGGACGTAGAACAAAGTCAATTGATGGAGGTTCTGAGACTGCACTGTCTCCACTACACCGTTGGTCATGAATCCGACCTCTCCG
 T Q K H K K H S V L H L V P V N I T S K D S D V T E V M W Q P V L R R G R G
 CTGGAGGCCCAGGAGACATTGTACGAGTCTGGGACACTGGAAATTTATCTGCTCTATAGTCAGTCTCTGTTTCATGTGCTTTTCAAAATGGGTCAAGTGGTATCTCGGGAGGA
 GACCTCCGGTCCCTCTGTAAACATGCTCAGACCTGTGACCTTAATATAGACGAGATATCAGTCCAGGACAAAGTACTACACTGAAAGTGTACCCAGTCCACCATAGAGCCCTTCCT
 L E A Q G D I V R V W D T G I Y L L Y S Q V L F H D V T P T M Q Q V V S R E G
 CAAGGAGAGAGAACTCTATTCCGATGTATCAGAAATGTCCTTCTGATCTGACCGTGTCAATAGCTGTACAGTGCAGGTGTCTTTCATTATCATCAAGGGATATTATC
 GTTCCCTCTCTCTTTGAGATAAGGCTACATAGTCTTCAACGGAAGACTAGGACTGGCACGGATGTTATCGAGCATGTCCACGTCCACAGAAAGTAAATGTAGTTCCTTATATAG
 Q G R R E T L F R C I R S M P S D P D R A Y N S C Y S A Q V F H L H Q G D I I
 ACTGTCAAATTCACGGGCAACGCAAACTTAGCCTTTCTCGCATGGAACATTCTCGGGTTTGTGAAACTATGA
 TGACAGTTTAAAGTGCCGTTTTCGTTTGAATCGAAGAGGGGTACTCTTGAAGACCCCAACACTTGTACT
 T V K I P R A N A K L S L S P H G T P L G P V X L

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FIG. 8A

purified myc murine
APRIL protein binds to
APRIL-R2 transfected
293 cells

*FIG. 8B**FIG. 8C*

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FIG. 9

FLAG Tagged Human APRIL (soluble)

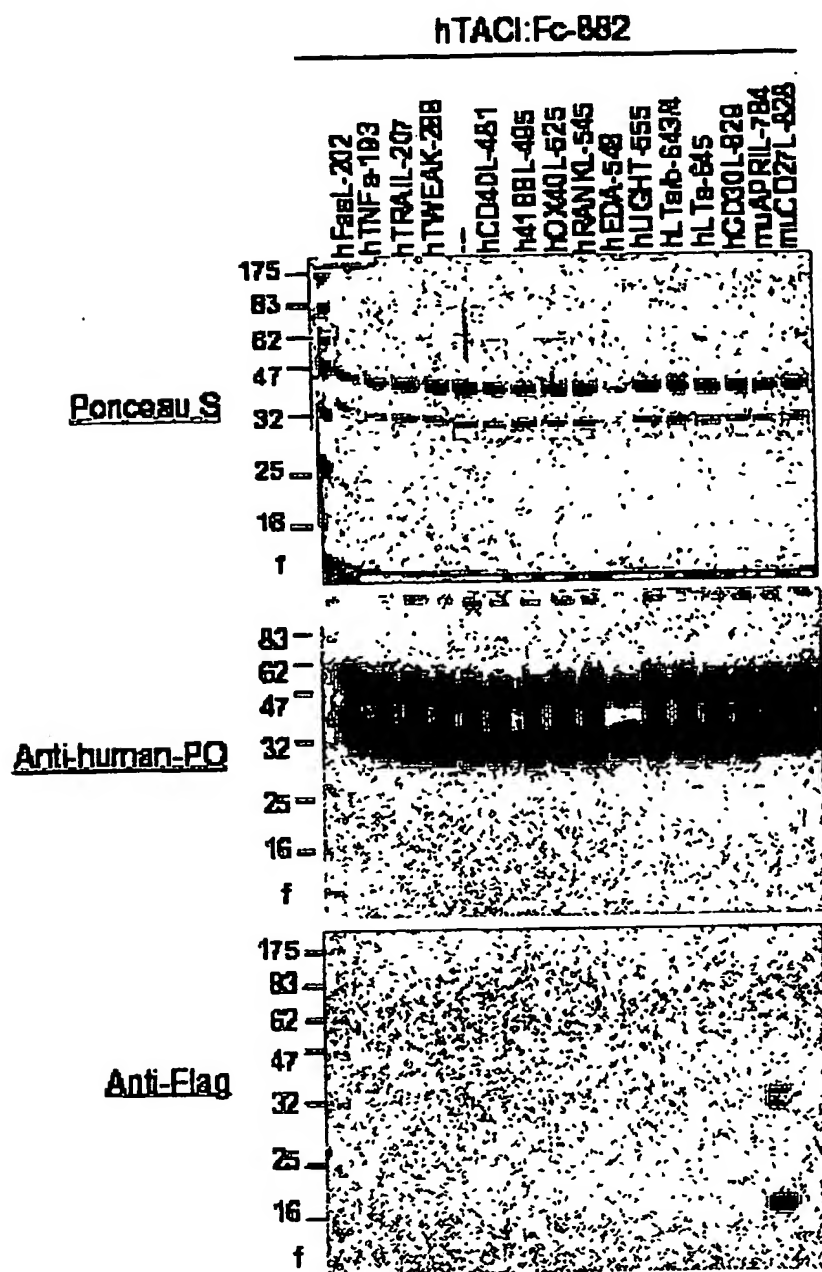
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1  ATG GCT ATC ATC TAC CTC ATC CTC CTG TTC ACC GCT GTG CGG GGC GAT TAC
   >M A I I I Y L I L L F T A V R G D Y
52  AAA GAC GAT GAC GAT AAA GGA CCC GGA CAG GTG CAG CTG GCA GTG CTC
   >K D D D K K G P G Q V Q L Q > A V L
103 ACC CAA AAA CAG AAG AAG CAG CAC TCT GTC CTC CAC CTG GTT CCC ATT AAC
   >T Q K Q K K Q K Q H S V L H L V P I N
154 GCC ACC TCC AAG GAT GAC TCC GAT GTG ACA CAG GTG ATG TGG CAA CCA GCT
   >A T S K D D S D V T E V M W Q P A
205 CTT AGG CGT GGG AGA GGC CTA CAG GCC CAA GGA TAT GGT GTC CGA ATC CAG
   >L R R G R G L Q A Q G Y G V R I Q
256 GAT GCT GGA GTT TAT CTG CTG TAT AGC CAG GTC CTG TTT CAA GAC GTG ACT
   >D A G V Y L L Y S Q V L F Q D V T
307 TTC ACC ATG GGT CAG GTG GTG TCT CGA GAA GGC CAA GGA AGG CAG GAG ACT
   >F T M G Q V V S R E G Q G R Q E T
358 CTA TTC CGA TGT ATA AGA AGT ATG CCC CAC CCG GAC CGG GCC TAC AAC
   >L F R C I R S M P S H P D R A Y N
409 AGC TGC TAT AGC GCA GGT GTC TTC CAT TTA CAC CAA GGG GAT ATT CTG AGT
   >S C Y S A Q V F H L H Q G D I L S
460 GTC ATA ATT CCC CGG GCA AGG GCG AAA CTT AAC CTC TCT CCA CAT GGA ACC
   >V I I I P R A R A K L N L S P H G T
511 TTC CTG GGG TTT GTG AAA CTG
   >F L G G P V K L

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FIG. 10

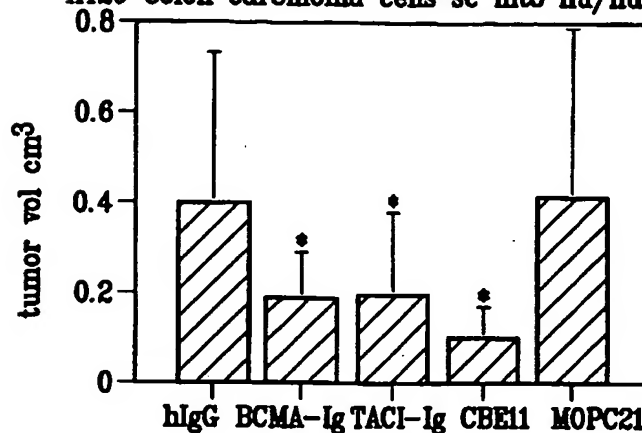
Co-IP of APRIL with TACI-HlgG1

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FIG. 11

Tumor growth 26 days after implantation of HT29 colon carcinoma cells sc into nu/nu mice

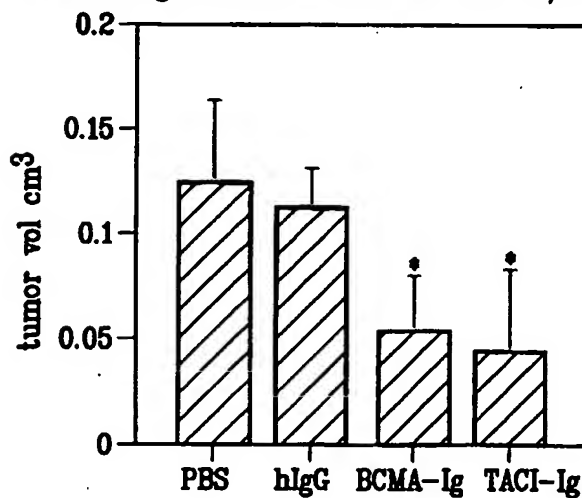


*: p<0.05

hlgG and MOPC21 are controls for the receptor-Ig fusion proteins and for the positive control mAb CBE11, respectively.

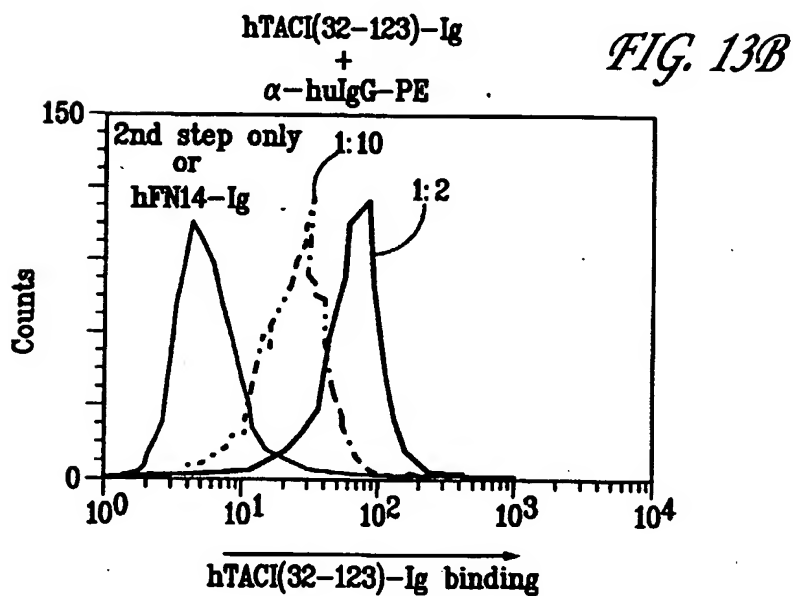
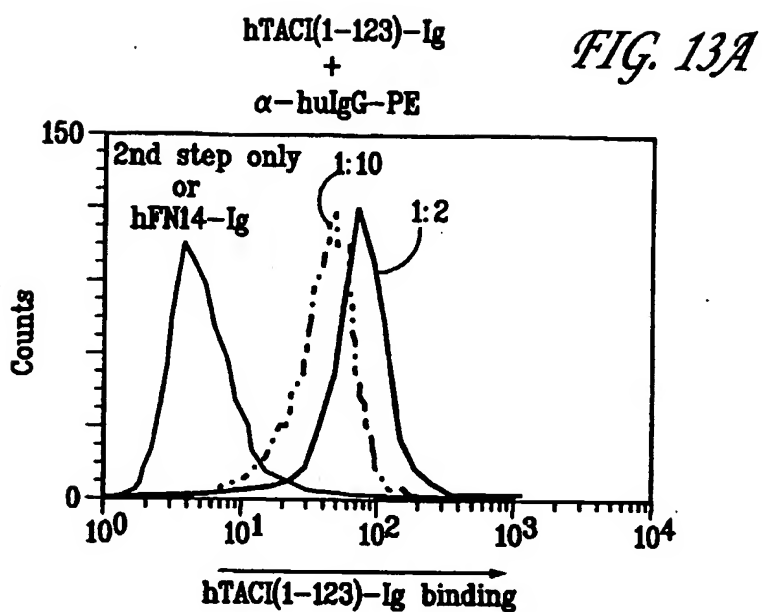
FIG. 12

Tumor growth 41 days after implantation of A549 lung carcinoma cells sc into nu/nu mice



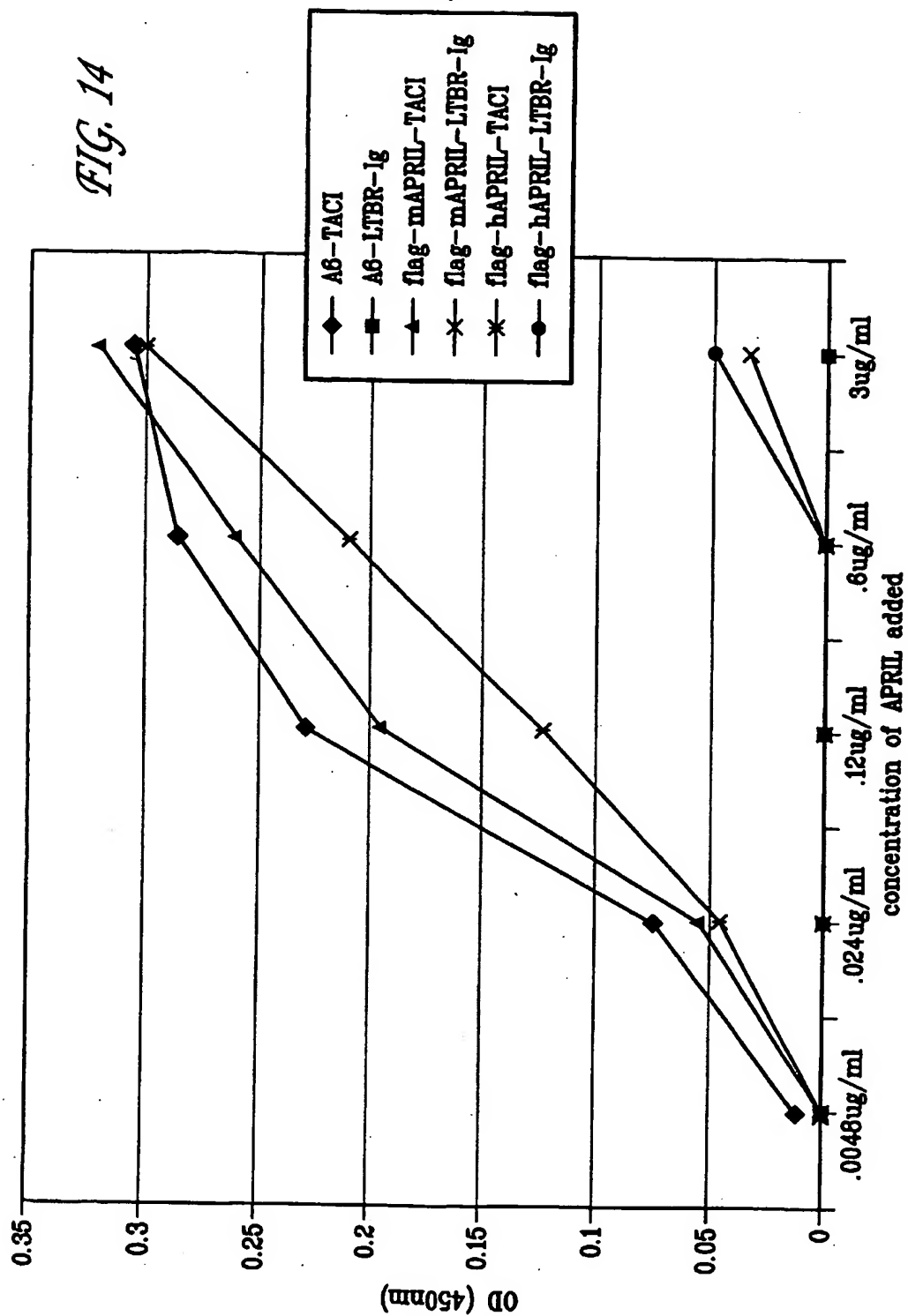
*: p<0.001

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FIG. 14



(19) World Intellectual Property Organization
International Bureau



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1 November 2001 (01.11.2001)

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(74) Agents: MILLER, Suzanne, E. et al.: Woodcock Washburn LLP, 46th floor, One Liberty Place, Philadelphia, PA 19103 (US).

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(71) Applicants (*for all designated States except US*): BIOGEN, INC. [US/US]; 14 Cambridge Centre, Cambridge, MA 02142 (US). APOTECH R & D S.A. [CH/CH]; 84, rue du Rhône, CH-1204 Genève (CH).

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): AMBROSE, Christine [US/US]; 197 Wakefield Street, Reading, MA 01867 (US). THOMPSON, Jeffrey [US/US]; 60 Newcomb Road, Stoneham, MA 02180 (US). SCHNEIDER, Pascal [CH/CH]; Tuileries 7, Ch-1066 Epalinges (CH). RENERT, Paul [US/US]; 278 Ridge Street, Millis, MA 02054 (US).

Declarations under Rule 4.17:

- of inventorship (Rule 4.17(iv)) for US only
- of inventorship (Rule 4.17(iv)) for US only
- of inventorship (Rule 4.17(iv)) for US only
- of inventorship (Rule 4.17(iv)) for US only

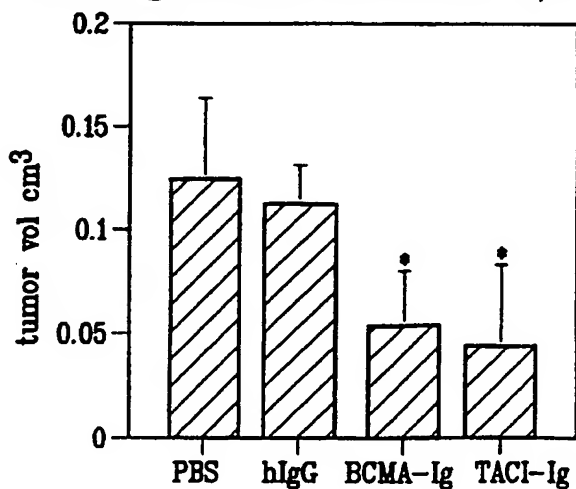
Published:

- with international search report

[Continued on next page]

(54) Title: USE OF TACI AS AN ANTI-TUMOR AGENT

Tumor growth 41 days after implantation of A549 lung carcinoma cells sc into nu/nu mice



*: p<0.001

(57) Abstract: A method of treating a mammal for a condition associated with undesired cell proliferation comprising administering to said mammal an effective amount of a TACI reagent, wherein said reagent extends mean survival time of said mammal by about 10 % or more as compared to the absence of administering the TACI reagent.

WO 01/81417 A3



(88) Date of publication of the international search report:
20 June 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Internatic Application No

PCT/US 01/40626

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/705 C12N15/12 C12N15/62 C07K19/00 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, GENSEQ, EMBL, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	CUBILLOS SUZANA ET AL: "Effect of blocking TNF on IL-6 levels and metastasis in a B16-BL6 melanoma/mouse model." ANTICANCER RESEARCH, vol. 17, no. 3C, 1997, pages 2207-2211, XP001051935 ISSN: 0250-7005 page 2210, left-hand column; figure 5 -/--	1-20

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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O document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search

10 January 2002

Date of mailing of the international search report

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Authorized officer

ALCONADA RODRIG..., A

INTERNATIONAL SEARCH REPORT

Internat'l Application No

PCT/US 01/40626

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	BROWNING J L ET AL: "SIGNALING THROUGH THE LYMPHOTOXIN BETA RECEPTOR INDUCES THE DEATH OF SOME ADENOCARCINOMA TUMOR LINES" JOURNAL OF EXPERIMENTAL MEDICINE, TOKYO, JP, vol. 183, no. 3, 1 March 1996 (1996-03-01), pages 867-878, XP000571445 ISSN: 0022-1007 page 869; table 1 ---	1-20
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P,X	WO 00 58362 A (HUMAN GENOME SCIENCES INC ;BAKER KEVIN (US); RUBEN STEVEN M (US);) 5 October 2000 (2000-10-05) page 58, line 5-24 page 73, line 15 -page 74, line 15 page 213, line 21 -page 214, line 16 ---	1-20
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Information on patent family members

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